

Serial No. New US Patent Application

Docket No. KC-0148

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Original) A method of determining the presence of a toxicant in a test sample, comprising the steps of;
 - exposing a eukaryote that has been transformed with a light emitting Ca^{2+} regulated photoprotein gene to a test sample
 - measuring the light produced by the transformed cell/organism
 - determining whether the amount of light is above or below a defined threshold at the time of exposure.
- 2 (Original) A method as in Claim 1 wherein the eukaryote is a fungi.
3. (Original) A method as in Claim 2 wherein the fungi is a filamentous fungi.
4. (Currently Amended) A method as in ~~Claims 2 or 3~~Claim 2 wherein the fungi is of the Aspergillus species.
5. (Original) A method as in Claim 1 wherein the eukaryote is a mammalian cell.
6. (Original) A method as in Claim 1 wherein the eukaryote is a plant cell.
7. (Currently Amended) A method as in ~~any of the previous Claims~~Claim 1 wherein the test sample comprises a toxicant.

8. (Currently Amended) A method as in ~~any of the previous Claims~~Claim 1 wherein the light emitting Ca^{2+} regulated photoprotein gene is a recombinant gene.

9. (Currently Amended) A method as in ~~any of the previous Claims~~Claim 1 wherein the light emitting Ca^{2+} regulated photoprotein gene is selected from the group comprising;

- aequorin gene
- halistaurin (mitrocomin) gene
- phialidin (clytin) gene
- obelin gene
- mnemiopsin gene
- berovin gene

10. (Currently Amended) A method as in ~~any of the previous Claims~~Claim 1 wherein the light emitting Ca^{2+} regulated photoprotein gene may be a functional homologue of a gene selected from the group comprising;

- aequorin gene
- halistaurin (mitrocomin) gene
- phialidin (clytin) gene
- obelin gene
- mnemiopsin gene
- berovin gene

11. (Currently Amended) A method as in ~~any of the previous Claims~~Claim 1 wherein the light emitting Ca^{2+} regulated photoprotein gene is an aequorin gene.

12. (Currently Amended) A method as in ~~any of the previous Claims~~Claim 1 wherein the light emitting Ca^{2+} regulated photoprotein gene is a recombinant aequorin gene.

13. (Currently Amended) A method as in ~~any of the previous Claims~~Claim 1 wherein the light that is measured is in the form of luminescence.

14. (Currently Amended) A method as in ~~any of the previous Claims~~Claim 1 wherein the test sample is added in advance of the application of a stimulus to the test sample.

15. (Original) A method as in Claim 14 wherein the stimulus is at least one or more from the group comprising; mechanical perturbation, hypo-osmotic shock, change in external calcium chloride concentration, temperature shock and pH shock.

16. (Currently Amended) A method as in ~~Claims 14 and 15~~Claim 14 wherein the test sample is added 1 minute to 1 prior to the application of the stimulus.

17. (Currently Amended) A method as in ~~Claims 14 to 16~~Claim 14 wherein the test sample is added 5 prior to the application of the stimulus.

18. (Currently Amended) A method as in ~~Claims 14 to 16~~Claim 14 wherein the test sample is added 30 minutes prior to the application of the stimulus.

19. (Original) A method of determining the presence of a toxicant in a test sample, comprising the steps of;

- exposing a eukaryote that has been transformed with a light emitting Ca^{2+} regulated photoprotein gene to a test sample
- measuring the light produced by the transformed cell/organism

- determining whether the amount of light is above a defined threshold at a specified time after the time of exposure.

20. (Original) A method as in Claim 19 which comprises the step of determining whether the amount of light is below a defined threshold.

21. (Currently Amended) A method as in ~~Claims 19 and 20~~Claim 19 wherein the specified time after the time of exposure is 11 minutes.

22. (Currently Amended) A method as in ~~Claims 19 to 24~~Claim 19 wherein the eukaryote is a fungi.

23. (Original) A method as in Claim 22 wherein the fungi is a filamentous fungi.

24. (Currently Amended) A method as in ~~Claims 22 to 23~~Claim 22 wherein the fungi is of the Aspergillus species.

25. (Currently Amended) A method as in ~~Claims 19 to 21~~Claim 19 wherein the eukaryote is a mammalian cell.

26. (Currently Amended) A method as in ~~Claims 19 to 21~~Claim 19 wherein the eukaryote is a plant cell.

27. (Currently Amended) A method as in ~~Claims 19 to 26~~Claim 19 wherein the test sample comprises a toxicant.

28. (Currently Amended) A method as in ~~Claims 19 to 27~~Claim 19 wherein the light emitting Ca²⁺ regulated photoprotein gene is a recombinant gene.

29. (Currently Amended) A method as in ~~Claims 19 to 28~~Claim 19 wherein the light emitting Ca²⁺ regulated photoprotein gene is selected from the group comprising;

- aequorin gene
- halistaurin (mitrocomin) gene
- phialidin (clytin) gene
- obelin gene
- mnemiopsin gene
- berovin gene

30. (Currently Amended) A method as in ~~Claims 19 to 29~~Claim 19 wherein the light emitting Ca²⁺ regulated photoprotein gene may be a functional homologue of a gene selected from the group comprising;

- aequorin gene
- halistaurin (mitrocomin) gene
- phialidin (clytin) gene
- obelin gene
- mnemiopsin gene
- berovin gene

31. (Currently Amended) A method as in ~~Claims 19 to 30~~Claim 19 wherein the light emitting Ca²⁺ regulated photoprotein gene is an aequorin gene.

32. (Currently Amended) A method as in ~~Claims~~ Claim 31 wherein the light emitting Ca^{2+} regulated photoprotein gene is a recombinant aequorin gene.

33. (Currently Amended) A method as in ~~Claims 19 to 32~~ Claim 19 wherein the light that is measured is in the form of luminescence.

34. (Currently Amended) A method as in ~~Claims 19 to 33~~ Claim 19 wherein the test sample is added in advance of the application of a stimulus to the test sample.

35. (Original) A method as in Claim 34 wherein the stimulus is at least one or more from the group comprising; mechanical perturbation, hypo-osmotic shock, change in external calcium chloride concentration, temperature shock and pH shock.

36. (Currently Amended) A method as in ~~Claims 34 to 35~~ Claim 34 wherein the test sample is added 1 minute to 1 prior to the application of the stimulus.

37. (Currently Amended) A method as in ~~Claims 34 to 36~~ Claim 34 wherein the test sample is added 5 minutes prior to the application of the stimulus.

38. (Currently Amended) A method as in ~~Claims 34 to 36~~ Claim 34 wherein the test sample is added 30 minutes prior to the application of the stimulus.

39. (Original) A method of determining the presence of a toxicant in a test sample, comprising the steps of;

- exposing a eukaryote that has been transformed with a light emitting Ca^{2+} regulated photoprotein gene to a test sample
- measuring the light produced by the transformed cell/organism

- and comparing the light measurement data with a bank of known toxicity reference data.

40. (Original) A method as in Claim 39 wherein the method comprises the step of determining whether the amount of light is below a defined threshold.

41. (Currently Amended) A method as in ~~Claims 39 to 40~~Claim 39 wherein the specified time after the time of exposure is 11 minutes.

42. (Currently Amended) A method as in ~~Claims 39 to 40~~Claim 39 wherein the eukaryote is a fungi.

43. (Original) A method as in Claim 42 wherein the fungi is a filamentous fungi.

44. (Currently Amended) A method as in ~~Claims 42 to 43~~Claim 42 wherein the fungi is of the *Aspergillus* species.

45. (Currently Amended) A method as in ~~Claims 39 to 41~~Claim 39 wherein the eukaryote is a mammalian cell.

46. (Currently Amended) A method as in ~~Claims 39 to 44~~Claim 39 wherein the eukaryote is a plant cell.

47. (Currently Amended) A method as in ~~Claims 39 to 46~~Claim 39 wherein the test sample comprises a toxicant.

48. (Currently Amended) A method as in ~~Claims 39 to 47~~Claim 39 wherein the light emitting Ca²⁺ regulated photoprotein gene is a recombinant gene.

49. (Currently Amended) A method as in ~~Claims 39 to 48~~Claim 39 wherein the light emitting Ca²⁺ regulated photoprotein gene is selected from the group comprising;

- aequorin gene
- halistaurin (mitrocomin) gene
- phialidin (clytin) gene
- obelin gene
- mnemiopsin gene
- berovin gene

50. (Currently Amended) A method as in ~~Claims 39 to 49~~Claim 39 wherein, the light emitting Ca²⁺ regulated photoprotein gene may be a functional homologue of a gene selected from the group comprising;

- aequorin gene
- halistaurin (mitrocomin) gene
- phialidin (clytin) gene
- obelin gene
- mnemiopsin gene
- berovin gene

51. (Currently Amended) A method as in ~~Claims 39 to 50~~Claim 39 wherein the light emitting Ca²⁺ regulated photoprotein gene is an aequorin gene.

52. (Currently Amended) A method as in ~~Claims 39 to 51~~Claim 39 wherein the light emitting Ca^{2+} regulated photoprotein gene is a recombinant aequorin gene.

53. (Currently Amended) A method as in ~~Claims 39 to 52~~Claim 39 wherein the light that is measured is in the form of luminescence.

54. (Currently Amended) A method as in ~~Claims 39 to 53~~Claim 39 wherein the test sample is added in advance of the application of a stimulus to the test sample.

55. (Original) A method as in Claim 54 wherein the stimulus is at least one or more from the group comprising; mechanical perturbation, hypo-osmotic shock, change in external calcium chloride concentration, temperature shock and pH shock.

56. (Currently Amended) A method as in ~~Claims 54 to 55~~Claim 54 wherein the test sample is added 1 minute to 1 prior to the application of the stimulus.

57. (Currently Amended) A method as in ~~Claims 54 to 56~~Claim 54 wherein the test sample is added 5 minutes prior to the application of the stimulus.

58. (Currently Amended) A method as in ~~Claims 54 to 55~~Claim 54 wherein the test sample is added 30 minutes prior to the application of the stimulus.

59. (Currently Amended) A method as in ~~Claims 39 to 58~~Claim 39 wherein the method is used to determine the amount of toxicant in the sample.

60. (Currently Amended) A method as in ~~Claims 39 to 59~~Claim 39 wherein the method is used to identify the toxicant in the sample.

61. (Original) A method of determining the presence of a toxicant in a test sample, comprising the steps of;

- exposing a eukaryote that has been transformed with a light emitting Ca^{2+} regulated photoprotein gene to a test sample
- measuring the light produced by the transformed cell/organism
- converting the light data into a cytosolic free calcium ion concentration trace,
- and comparing at least one parameter of the cytosolic free calcium ion concentration trace with a bank of known toxicity reference data.

62. (Original) A method as in Claim 61 wherein the method comprises the step of determining whether the amount of light is below a defined threshold.

63. (Currently Amended) A method as in ~~Claims 61 to 62~~Claim 61 wherein the eukaryote is a fungi.

64. (Original) A method as in Claim 63 wherein the fungi is a filamentous fungi.

65. (Currently Amended) A method as in ~~Claims 63 to 64~~Claim 63 wherein the fungi is of the *Aspergillus* species.

66. (Currently Amended) A method as in ~~Claims 61 to 62~~Claim 61 wherein the eukaryote is a mammalian cell.

67. (Currently Amended) A method as in ~~Claims 61 to 62~~Claim 61 wherein the eukaryote is a plant cell.

68. (Currently Amended) A method as in ~~Claims 61 to 67~~ Claim 61 wherein the test sample comprises a toxicant.

69. (Currently Amended) A method as in ~~Claims 61 to 68~~ Claim 61 wherein the light emitting Ca²⁺ regulated photoprotein gene is a recombinant gene.

70. (Currently Amended) A method as in ~~Claims 61 to 69~~ Claim 61 wherein the light emitting Ca²⁺ regulated photoprotein gene is selected from the group comprising;

- aequorin gene
- halistaurin (mitrocomin) gene
- phialidin (clytin) gene
- obelin gene
- mnemiopsin gene
- berovin gene

71. (Currently Amended) A method as in ~~Claims 61 to 70~~ Claim 61 wherein the light emitting Ca²⁺ regulated photoprotein gene may be a functional homologue of a gene selected from the group comprising;

- aequorin gene
- halistaurin (mitrocomin) gene
- phialidin (clytin) gene
- obelin gene
- mnemiopsin gene
- berovin gene

72. (Currently Amended) A method as in ~~Claims 61 to 71~~Claim 61 wherein the light emitting Ca²⁺ regulated photoprotein gene is an aequorin gene.

73. (Currently Amended) A method as in ~~Claims 61 to 72~~Claim 61 wherein the light emitting Ca²⁺ regulated photoprotein gene is a recombinant aequorin gene.

74. (Currently Amended) A method as in ~~Claims 61 to 73~~Claim 61 wherein the light that is measured is in the form of luminescence.

75. (Currently Amended) A method as in ~~Claims 61 to 74~~Claim 61 wherein the test sample is added in advance of the application of a stimulus to the test sample.

76. (Original) A method as in Claim 75 wherein the stimulus is at least one or more from the group comprising; mechanical perturbation, hypo-osmotic shock, change in external calcium chloride concentration, temperature shock and pH shock.

77. (Currently Amended) A method as in ~~Claims 75 to 76~~Claim 75 wherein the test sample is added 1 minute to 1 prior to the application of the stimulus.

78. (Currently Amended) A method as in ~~Claims 75 to 77~~Claim 75 wherein the test sample is added 5 minutes prior to the application of the stimulus.

79. (Currently Amended) A method as in ~~Claims 75 to 77~~Claim 75 wherein the test sample is added 30 minutes prior to the application of the stimulus.

80. (Currently Amended) A method as in ~~Claims 61 to 79~~Claim 61 wherein light is measured for between 1 minute and 5 hours following the application of the stimulus.

81. (Currently Amended) A method as in ~~Claims 61 to 79~~Claim 61 wherein light is measured for between 1 minute and 96 hours following the application of the stimulus.

82. (Currently Amended) A method as in ~~Claims 61 to 79~~Claim 61 wherein light is measured for 5 minutes following the application of the stimulus.

83. (Currently Amended) A method as in ~~Claims 61 to 82~~Claim 61 wherein the cytosolic free calcium ion trace is a plot of the cytosolic free calcium ion concentration against time.

84. (Currently Amended) A method as in ~~Claims 61 to 83~~Claim 61 wherein the parameter is at least one or more selected from the group comprising;

- lag time
- rise time
- absolute amplitude
- relative amplitude
- Length of transient at 20%, 50% and 80% of maximum amplitude
- number of cytosolic free calcium ion concentration increases
- percentage increase in final cytosolic free calcium ion concentration resting level
- percentage increase in recovery time
- percentage increase in pre-stimulating cytosolic free calcium ion concentration resting level
- total concentration of calcium

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85. (Currently Amended) A method as in ~~Claims 61 to 84~~Claim 61 wherein the method is used to determine the amount of toxicant in the sample.

86. (Currently Amended) A method as in ~~Claims 61 to 85~~Claim 61 wherein the method is used to identify the toxicant in the sample.